Midyear report for North Dakota Soybean Council (July 1, 2023 to November 30, 2023)

a. Research Project Title, Principal and Co-Investigators

Title: Understanding How Fusarium Affects Soybean in North Dakota and Development of Disease Management Strategies

Principal Investigator: Febina Mathew (North Dakota State University, Department of Plant Pathology

Co-Investigators: Richard Webster, Samuel Markell, Carrie Miranda, Guiping Yan, Thomas Baldwin, and Joao Paulo Flores

b. Research Overview and Objectives

Research Overview: Seedling diseases, caused by *Fusarium*, can be a major problem in U.S. soybean production. In North Dakota, sudden death syndrome (SDS), which is caused by *Fusarium virguliforme*, was confirmed in 2020, and two other species of *Fusarium*, *F. solani,* and *F. tricinctum*, have been implicated in causing root rot. The ND soybean farmers have limited options to manage *Fusarium* diseases, which include the use of a few fungicide seed treatments, and varieties with tolerance to the SDS fungus, *F. virguliforme*. Currently, the distribution of *Fusarium* species (including SDS) and other seedling organisms such as species of *Rhizoctonia* and *Pythium* in ND is not understood. However, these diseases are occurring in pockets in ND soybean fields, and there may be several reasons for their emergence. Soybeans may be planted year after year because of higher market prices. Farmers may adopt no-till or reduced tillage practices, which leaves crop residues infested with *Fusarium* on or near the soil surface. Additionally, it is possible that *Fusarium* diseases are showing up in fields where soybean cyst nematode (SCN) may be present. Thus, in the proposed study, we developed these objectives: (1) Characterize the species distribution of *Fusarium* associated with soybean; (2) Characterize the pathogenicity of *Fusarium* species; (3) Determine how the presence of SCN can affect the development of SDS; and (4) Determine the impact of seed treatment on *Fusarium*. The information obtained from this study will complement NDSU's efforts to help and educate farmers to manage SCN, SDS, and other *Fusarium* diseases with effective disease management strategies.

c. Completed Work: Deliverables and/or Milestones.

- Eight species of *Fusarium* (*F. solani, F. acuminatum, F. caucasicum, F. oxysporum, F. curvatum, F. serpentinum, F. clavus, F. communae*) have been isolated from soil samples collected from 75 fields in addition to *Rhizoctonia* and *Pythium* spp., which have been reported in the U.S. to cause seedling diseases of soybean.
- Among the species of *Fusarium, F. caucasicum, F. curvatum,* and *F. clavus* have not been reported on soybean previously in the U.S.
- For the first time, *Phytopythium* species were identified in ND.

- Results from the seed treatment trials show greater soybean yield with products such as ILEVO, Saltro, and CeraMax (11 to 17% yield increase) when compared to non-treated control.

d. Progress of Work and Results to Date

Objective 1. Characterize the species distribution of *Fusarium* associated with soybean (Objective in progress)

Mathew's lab and Webster's lab conducted a soybean disease survey across 30 counties (3-5 fields per county, Fig. 1) at the vegetative (May – June) or reproductive growth stages (August – September) of soybeans to collect soil samples and infected plant samples.

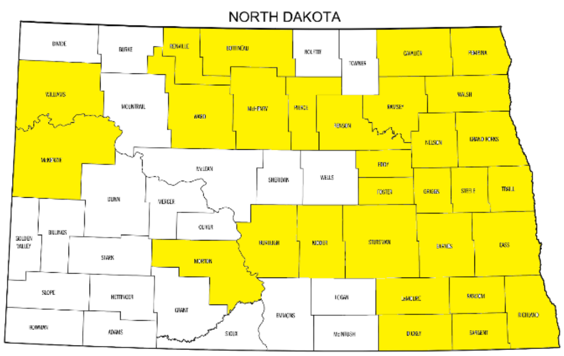


Fig. 1. North Dakota counties surveyed in 2023.

For fungal isolation, about 20 grams of homogenized soil from each field was moistened to 60 to 70 percent and baited with a single 7-day-old Barnes seedling. For each field, six replications were maintained. After 10 days of incubation, the infected roots were surface sterilized in 0.5 % bleach solution and 70 % ethanol for 30 sec, rinsed 2 times in sterile distilled water, and blotted dry. Root pieces were placed in antibiotic-amended (0.06% streptomycin sulfate) half-strength potato dextrose agar (PDA) media at 24± 2°C for 14 days under diffused light conditions. The fungal isolates were purified by hyphal tipping and morphological characterization was done based on colony appearance and spore production on PDA. Based on morphology, isolates were grouped, and representative samples were subjected to molecular characterization and identification using Internal Transcribed Spacer (ITS) gene sequencing for the *Rhizoctonia* or *Pythium* genus and Translation elongation factor 1 (EF1) gene sequencing for the *Fusarium* genus and the identity of the fungal organisms was confirmed using the type sequences available in the Mycobank database. About 461 isolates of seedling pathogens were recovered from 75 fields where *Fusarium* sp. and *Rhizoctonia* sp. added up to 58% of the total pathogen population, whereas the frequency of Pythium sp. was close to 20% (Fig. 2). At least eight species of Fusarium (accounting for 50 % of the total pathogens) were obtained, which include *F. solani* species complex, *Neocosmospora solani* (syn. the *F. solani* species complex, FSSC), *F. acuminatum (*syn. *F. tricinctum* species complex), *F. caucasicum*, *F.*

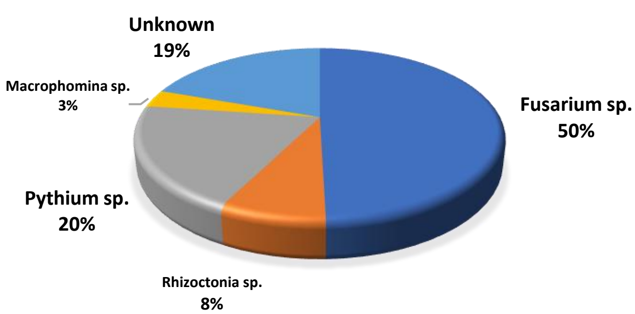


Fig. 2. Seedling pathogens characterized from the 2023 survey (*n*= 75 fields).

oxysporum, *F. curvatum* (syn. *F. oxysporum* species complex), *F. serpentinum*, *F. clavus* (syn. F. *incarnatum-equiseti* species complex), and *F. communae*. In addition, a difference in species distribution was observed across counties (Fig. 3).

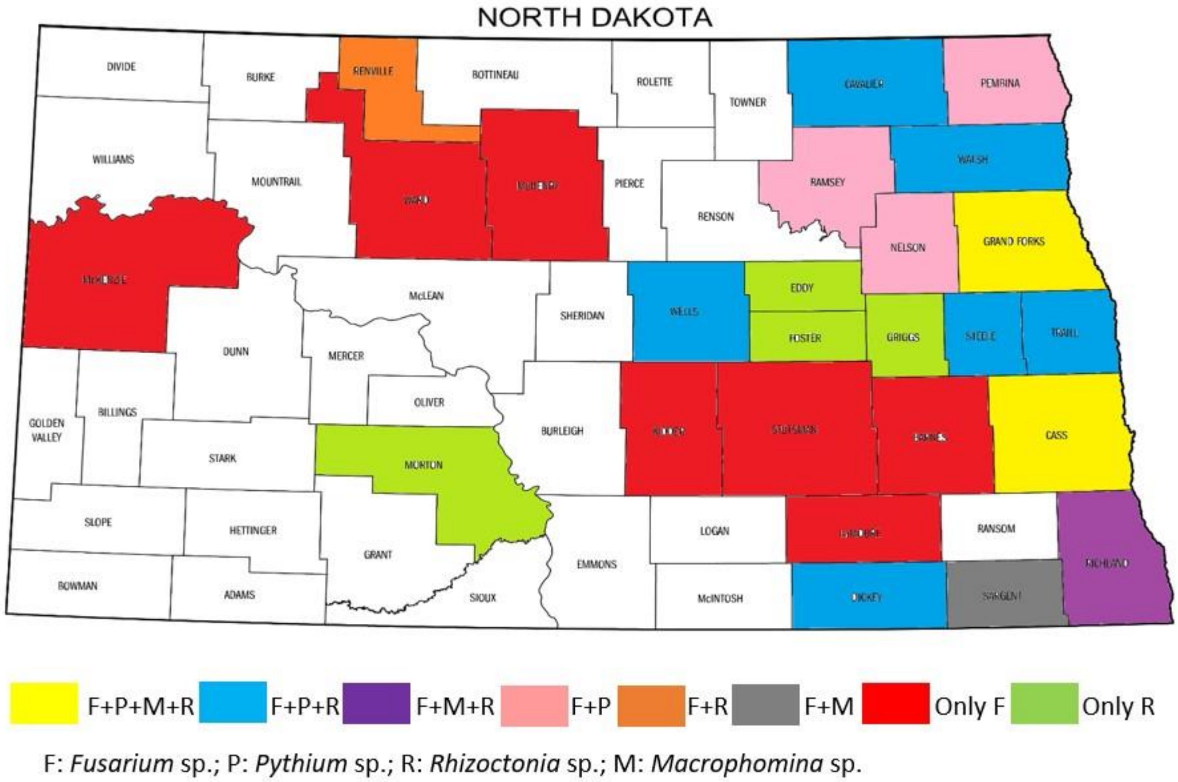


Fig. 3. Distribution of seedling pathogens by ND county from the 2023 survey (*n*=75 fields).

Objective 2. Characterize the pathogenicity of *Fusarium* species. (Objective in progress)

This objective was split into two sub-objectives: (1) Screen soybean accessions for root infection of *F. virguliforme* using an inoculum layer technique, and **(2)** Study cross-pathogenicity of isolates of *Fusarium* from soybean and wheat on both crops in the greenhouse using the layer (soybean) and point inoculation (wheat) techniques.

For sub-objective 1, Mathew's lab set up a preliminary experiment with a single isolate of *Fusarium virguliforme* (obtained from Iowa State University). The experiment was set up in a completely randomized design using two varieties ('Spencer' SDS susceptible and 'Ripley' SDS resistant) in the greenhouse, and the layer inoculation method was used. Plastic cups were used which were filled with 80 grams of potting mix, 12 grams of fungal inoculum, followed by 20 grams of potting mix, into which three seeds of Spencer or Ripley were planted and covered with 20 grams of potting mix (inoculum density was 1:10). Throughout the experiment, the temperature was maintained at 23 + 2°C and humidity of 60 to 70% in the greenhouse, and the seedlings were watered every alternate day (water temperature at watering was 17°C and the potting mix was moistened to either 80% or 100% water holding capacity). After 14 days of incubations, seedlings were harvested, roots were washed, and ratings were taken to observe disease symptoms. It was observed that for the SDS-susceptible ‘Spencer’, more than 50% of the seeds decayed from fungal infection, regardless of whether the potting mix was moistened to 80 or 100 percent water holding capacity. In contrast, for the SDS-resistant ‘Ripley’, the seeds decayed when the potting mix was maintained at 100% water holding capacity, and the plants developed root rot infection when 80% water holding capacity was used (Fig. 4).

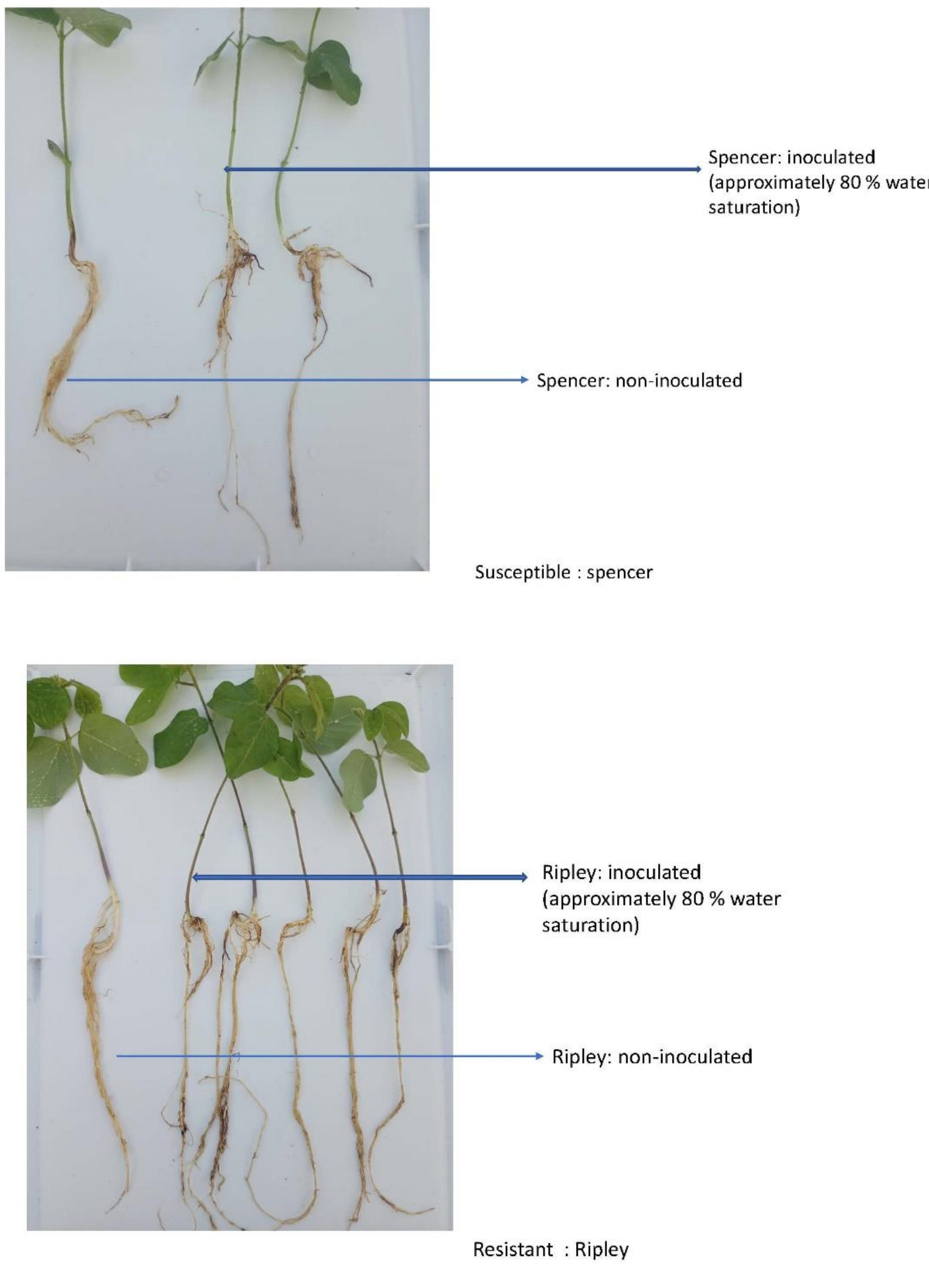


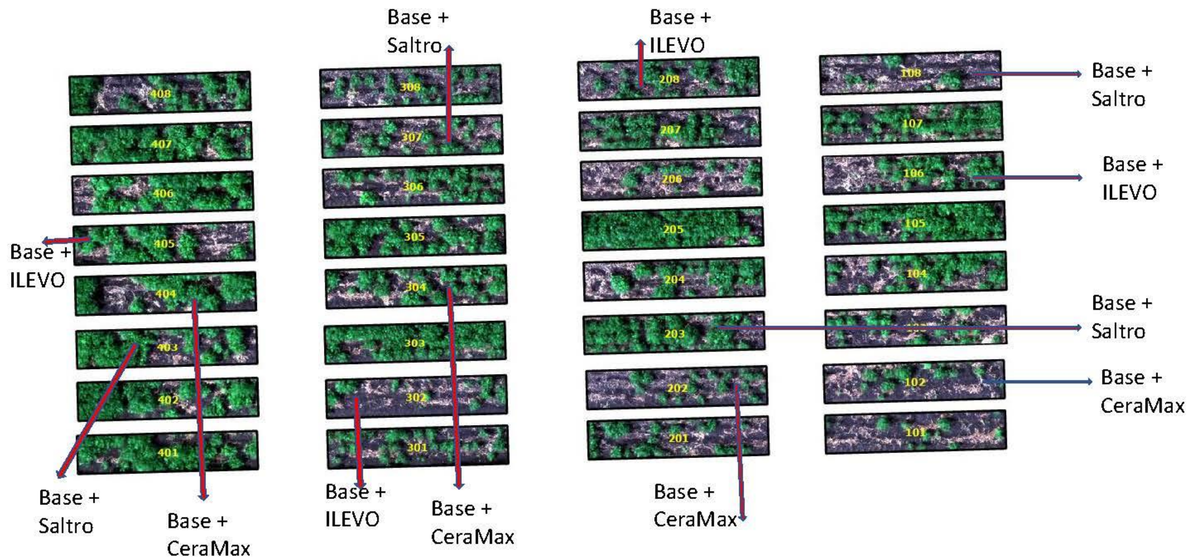
Fig. 4. Seedlings of cv. Ripley and Spencer were infected with *Fusarium virguliforme*.

Objective 4. Determine the impact of seed treatment on *Fusarium*. (Objective completed)

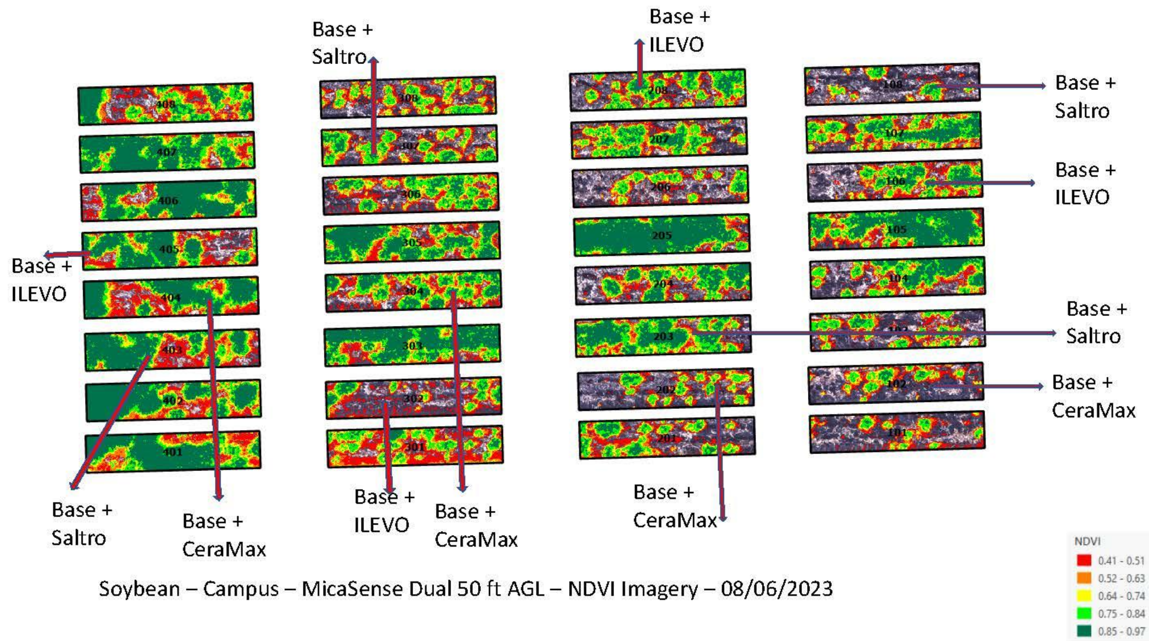
The trial was planted at the NDSU's Main Research Station in Fargo, ND by Dr. Markell's crew and Dr. Webster. The trial was established on a randomized complete block design with seven treatments (Base, Base + ILEVO, Base + Saltro, Base + TBZ + Headsup + Biost + SAR, Base + Saltro + Ataplan, Base + CeraMax, Base + ILEVO + CeraMax, and a non-treated control) on a susceptible soybean variety and there were four replications per seed treatment. Each plot (replication) was about 20 ft. long, and 10 ft wide (>100,000 plants/A). The trial was not inoculated. Plant emergence was evaluated three weeks after planting, and the number of emerged plants was statistically compared with the non-treated control plots. We did not observe any significant differences in the number of plants per acre as indicated in Table 1. We used drone sensors (cameras available at NDSU) in collaboration with Flores's lab to assess crop response to seed treatments (Fig. 5) and calculated normalized difference vegetation index (NDVI) from the sensor data to assess the density of vegetation (Table 1). We took the drone readings three times during the season but did not observe significant differences in NDVI among treatments. However, we noticed that the vegetation was denser in August when compared to July (NDVI values close to 1 indicate dense vegetation) and certain products such as ILEVO, Saltro, and CeraMax provided greater NDVI values when compared to the non-treated control plots. Yield data was obtained at the end of the growing season (October), and we observed greater yield with products such as ILEVO, Saltro, and CeraMax (11 to 17% yield increase) when compared to non-treated control plots (Table 1).

Table 1. Effect of fungicide seed treatments on soybean yield in Fargo, ND

Treatments	Plant per acre	NDVI (07/16/2023)	NDVI (08/06/2023)	NDVI (08/26/2023)	Yield (bu/A)
Non-Treated	69696.0	0.4	0.8	0.7	23.5
Base	71874.0	0.5	0.7	0.7	23.6
Base + ILEVO	70567.2	0.5	0.8	0.8	26.7
Base + Saltro	65557.8	0.4	0.8	0.8	27.3
Base + TBZ + Headsup + Biost + SAR	71874.0	0.5	0.8	0.8	30.1
Base + Saltro + Ataplan	68607.0	0.4	0.7	0.7	21.3
Base + CeraMax	72309.6	0.5	0.8	0.8	28.5
Base + ILEVO + CeraMax	67953.6	0.5	0.8	0.8	26.2
CV	**13.8**	**9.0**	**5.4**	**5.9**	**21.5**
***P*-value**	**0.9**	**0.4**	**0.2**	**0.2**	**0.4**



Soybean – Campus – MicaSense Dual 50 ft AGL – RGB Composite – 08/06/2023



Soybean – Campus – MicaSense Dual 50 ft AGL – NDVI Imagery – 08/06/2023

Fig. 5. Drone images when the soybean plants in the field trial were in the flowering growth stage (R1 growth stage).

e. Work to be Completed.

Objective 1. Characterize the species distribution of *Fusarium* associated with soybean.

- Mathew's lab will complete the processing of soil samples collected from the remaining 25 fields to make it to a total of 100 fields.

Objective 2. Characterize the pathogenicity of *Fusarium* species.

- Mathew's lab is screening 50 soybean accessions for resistance to root rot caused by *Fusarium virguliforme* (This experiment is in progress)
- Baldwin's lab will be performing cross-pathogenicity of *Fusarium graminearum* isolates obtained from soybean roots on wheat to understand the underlying pathogenicity genes associated with the fungus. Mathew's lab has provided isolates to Baldwin's lab for the study. (This experiment is in progress).

Objective 3. Determine how the presence of SCN can affect the development of SDS.

- Mathew's lab in collaboration with Yan's lab will set up a greenhouse experiment to determine how the presence of SCN can affect the root rot caused by *Fusarium graminearum*. (This experiment will be set up in January)

f. Other relevant information: potential barriers to achieving objectives, risk mitigation strategies, or breakthroughs.

Fusarium virguliforme was not successfully isolated from the soil samples collected from the fields surveyed in 2023, and this may be because the fields that were surveyed may not have been at a possible risk of SDS development. This was communicated to NDSC, and we will continue the research by using either the most prevalent or a virulent species of *Fusarium* (*F. graminearum*) in place of the SDS fungus.

g. Summary

Seedling pathogens (*Fusarium*, *Rhizoctonia*, *Pythium*, etc.) are a major yield-limiting factor in the U.S. soybean production regions (loss of $9.84/A). In North Dakota, the survey conducted in 2023 (75 fields across 30 counties) with funding from the North Dakota Soybean Council indicated that the major seedling pathogens are the species *Fusarium*, *Rhizoctonia*, *Pythium*, and possibly *Macrophomina*. Among species of *Fusarium*, *F. solani, F. acuminatum, F. caucasicum, F. oxysporum, F. curvatum, F. serpentinum, F. clavus,* and *F. communae* were identified. In addition, for the first time, a species of *Phytopythium* was identified in ND, and three species of *Fusarium, F. caucasicum, F. curvatum,* and *F. clavus* were not previously reported in the U.S. We hypothesize that factors such as heavy rains (above-normal precipitation) across ND in 2023, tillage practices, and limited host resistance options may have contributed to the prevalence of seedling diseases. Considering management options for farmers are limited, fungicide seed treatments were evaluated, and the results show greater soybean yield with products such as ILEVO, Saltro, and CeraMax (11 to 17% yield increase) when compared to non-treated control. Our results highlight the role of *Fusarium* in causing soybean seedling disease and root rot in ND, and the information obtained from the seed treatment trials and screening soybean germplasm for disease resistance will be used for developing and refining disease management programs targeting *Fusarium*.